

Preparative separation of a binary compound mixture – recovery of pure compounds and solvent consumption

Application

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Abstract

This Application Note describes the separation of two compounds from a binary compound mixture using the Agilent 1100 Series purification system. The parameters purity, recovery, analysis run time, solvent consumption and liquid phase composition are monitored and their interrelation is explained. Further, we discuss how the analysis can be optimized with regard to these parameters.



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Introduction

Separation of discrete compounds from a mixture is a typical task in preparative liquid chromatography. The simplest task is isolating two compounds from a binary mixture, however, it is also possible to isolate pure compounds from more complex compound mixtures, such as natural product extracts¹, for example. The compounds to isolate are typically isomers or enantiomers^{2,3}.

In this Application Note we demonstrate the separation of two compounds from a binary compound mixture using the Agilent 1100 Series purification system. The method developed on an analytical column was scaled up to preparative scale and the compounds were separated in milligram quantities. The separation was done repeatedly, varying the composition of the liquid phase to achieve a separation with good recovery and purity with minimum solvent consumption.

Equipment

The system included two Agilent 1100 Series preparative pumps, an Agilent 1100 Series diode array detector, an Agilent 1100 Series column organizer and an Agilent 220 micro plate sampler modified for higher flow rates. The system was controlled using the Agilent ChemStation (revision A.08.04) and the micro plate sampling software (revision A.03.02).

Results and Discussion

Overloading of the analytical column

The separation of the binary compound mixture was first done on an analytical column. Separation was achieved isocratically with a water/ acetonitrile mixture (figure 1).

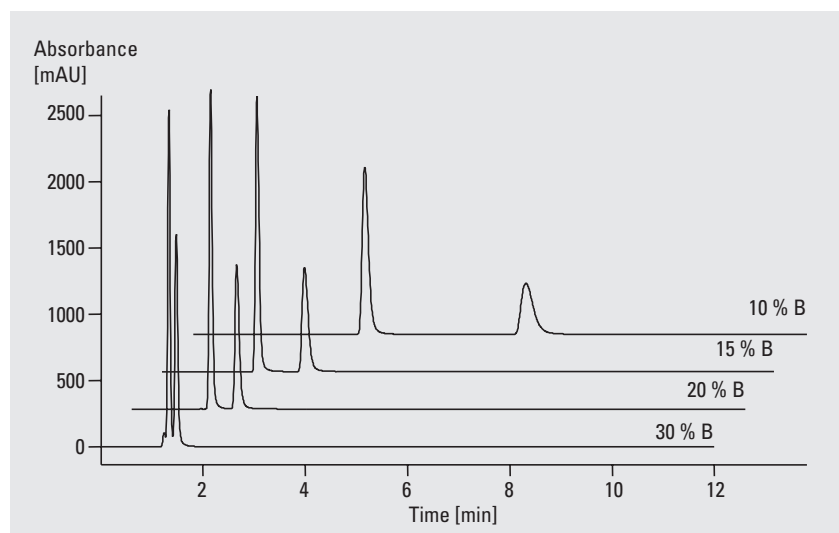


Figure 1
Analytical separation of binary compound mixtures

Mobile phase:	water = A acetonitrile = B
Isocratic:	between 10 and 30 % B
Stop time:	12 min
Column:	Zorbax SB-C18 3 x 150 mm, 5 µm
Flow:	0.6 ml/min
Injection:	5 µl
Column temperature:	ambient
UV detector:	DAD 270 nm/16 (reference 360 nm/100) Standard cell (10 mm pathlength)

Since the crude product was very soluble concentration overloading was possible. Figure 2 shows that the analytical column could be loaded with up to 500–1000 µg of each compound

Scale up to preparative scale

The scale-up from the analytical to the preparative column was calculated using the formulae shown in figure 3. After the first preparative run the flow rate was changed to 25 ml/min to achieve comparable retention times.

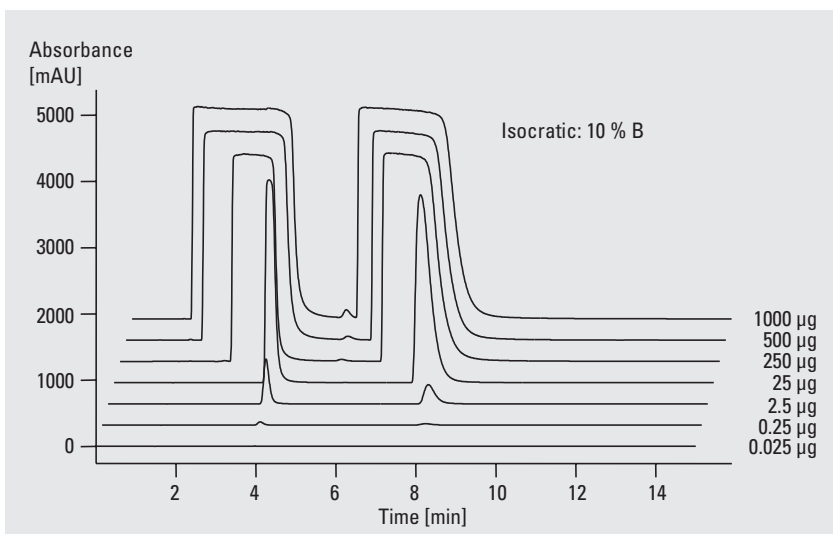


Figure 2
Overloading of the analytical column

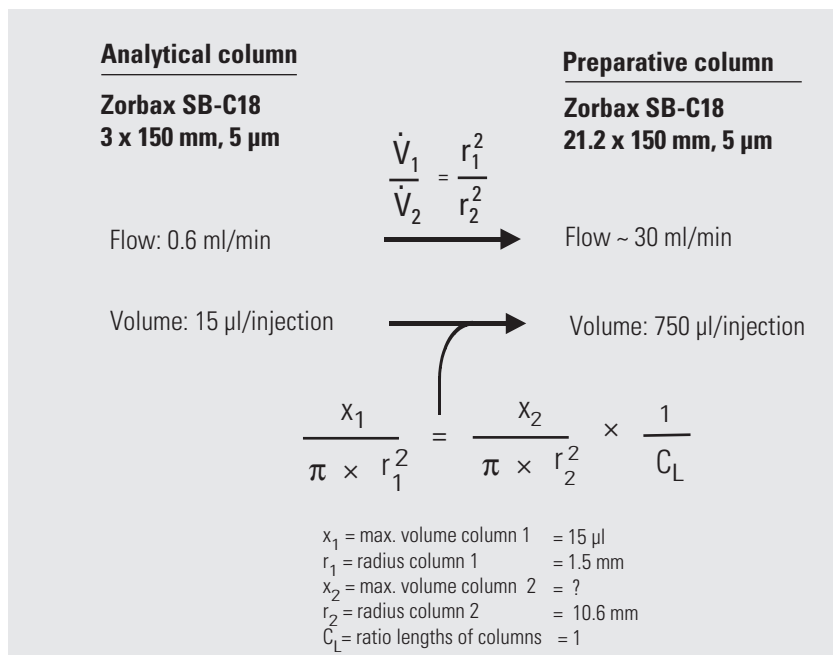


Figure 3
Scale up from analytical preparative column

Fractionation under isocratic conditions with 30 % B in the mobile phase

Figure 4a shows the preparative run and the fractionation based on retention time windows, figure 4b shows the fractionation result. It can be seen that only about 12 mg of compound 1 with a purity higher than 90 % could be isolated together with many fractions con-

taining the compound mixture. The overall recovery of the compounds was 24.5 mg of compound 1 (98 %) and 23.9 mg of compound 2 (94 %). The overall analysis run time could be minimized to about 2.6 minutes, that is, solvent consumption was about 65 ml per analysis.

Fractionation under isocratic conditions with 20 % B in the mobile phase

Figure 5a shows the preparative run and the fractionation based on retention time windows, figure 5b shows the fractionation result. About 21 mg of compound 1 with a purity higher than 95 % could be isolated, which is a recovery of 85 %. The isolated 23.5 mg of

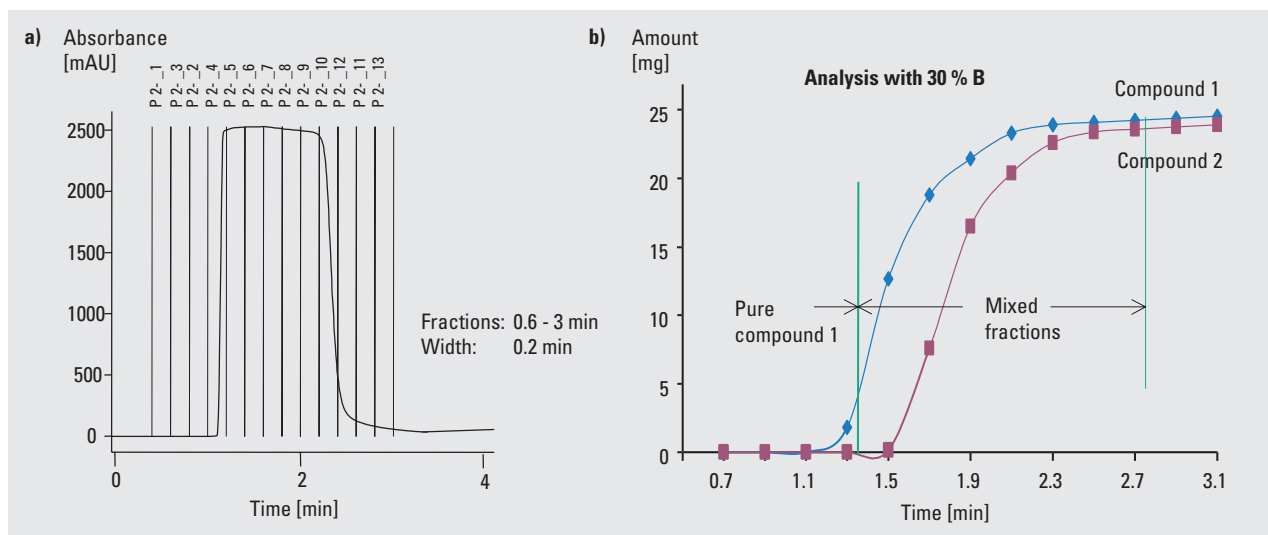


Figure 4
Fractionation with 30 % mobile phase B, b) isolation results, isocratic conditions

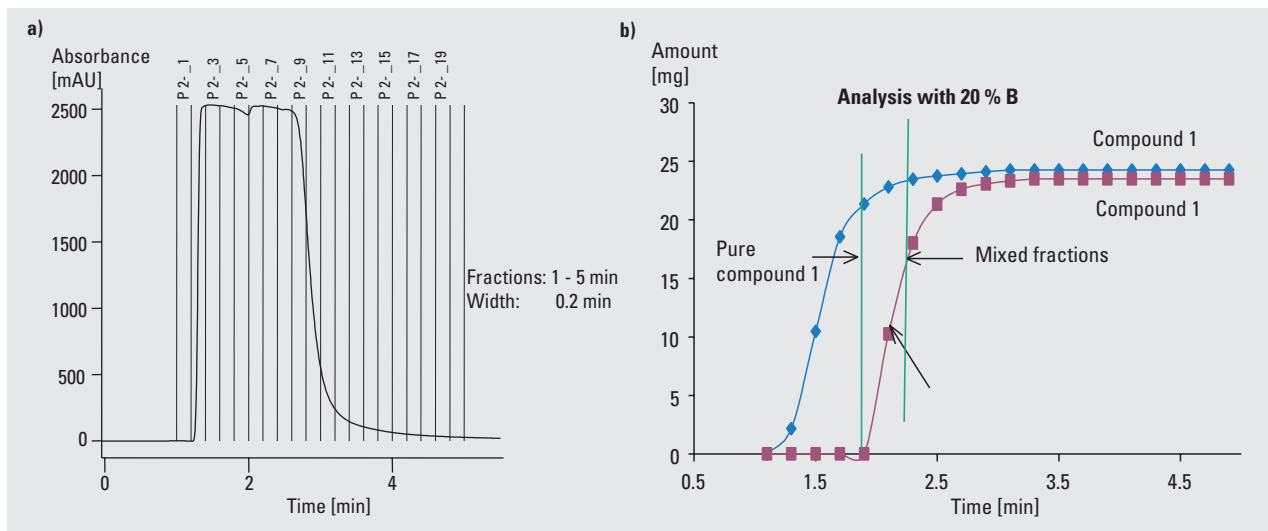


Figure 5
a) Isolation results, isocratic conditions, 20 % mobile phase B, b) isolation results, isocratic conditions

compound 2 (93 % recovery) had a purity of slightly less than 90 %. The overall recovery was 24.3 mg of compound 1 (97 %) and 23.5 mg of compound 2 (93 %). The overall analysis run time could be minimized to about 3.4 minutes, that is, solvent consumption was about 85 ml per analysis.

Fractionation under isocratic conditions with 15 % B in the mobile phase

Figure 6a shows the preparative run and the fractionation based on retention time windows, figure 6b shows the fractionation results. All isolated 24 mg of compound 1 had a purity higher than 98 %. The recovery was 97 %. The isolated 23 mg of compound 2 (90 % recovery) had a purity of 96 %. The overall analysis run time could be

minimized to about 4 minutes, that is, solvent consumption was about 100 ml per analysis.

Fractionation under isocratic conditions with 10 % B in the mobile phase

Figure 7a shows the preparative run and the fractionation based on retention time windows, figure 7b shows the fractionation results. 24.4 mg of compound 1 were

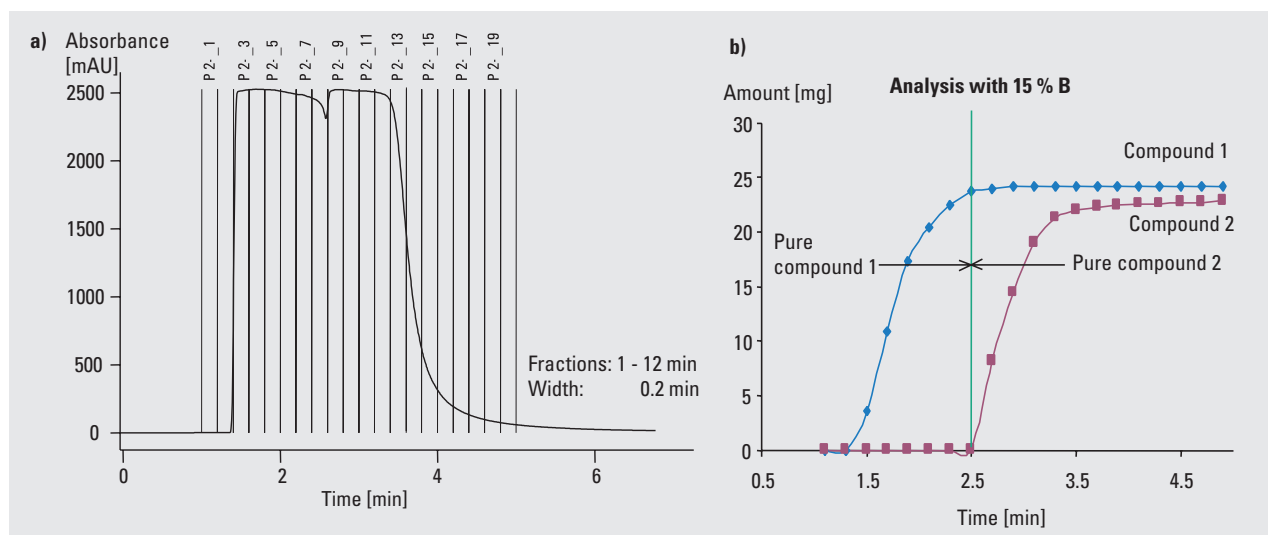


Figure 6
a) Fractionation with 15 % mobile phase B, b) isolation results, isocratic conditions

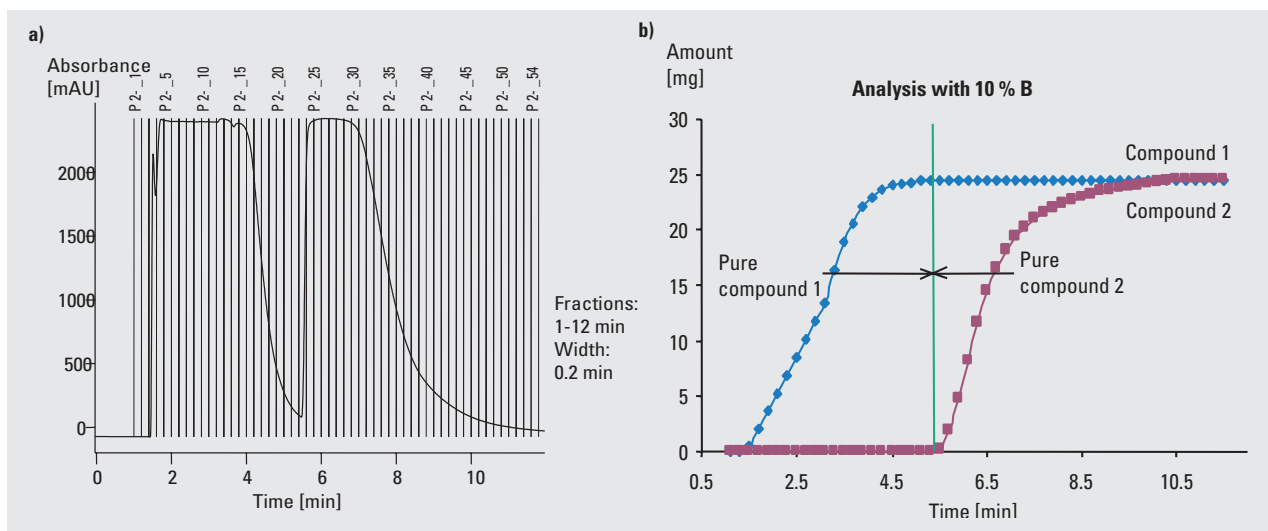


Figure 7
a) Fractionation with 10 % mobile phase B, b) isolation results, isocratic conditions

isolated with a purity of 98 % which is a recovery of 96 %. Compound 2 could be isolated with 97 % recovery (24.6 mg) and a purity of over 98 %. Despite these results the performance of the separation is not optimum. There are several fractions with no product between the fractions containing the pure compounds. The overall analysis run time of the separation is about 10.2 minutes, which means a solvent consumption of 255 ml per analysis.

Recovery against purity

Figures 8a and 8b show the recovered amount of the two compounds against purity. It can be clearly seen that the recovered amount with high purity increases with less acetonitrile in the mobile phase. Therefore, the analysis has to be adjusted depending on which parameter has higher priority. If high purity is required, for example for activity testing, and the recovery is not important, an analysis with more acetonitrile

can be done. Both compounds can be isolated with good purity with relative short run times. On the other hand, if good recovery is required but purity is less important, for example for isolation of intermediates in a synthesis sequence, less acetonitrile should be used in the mobile phase.

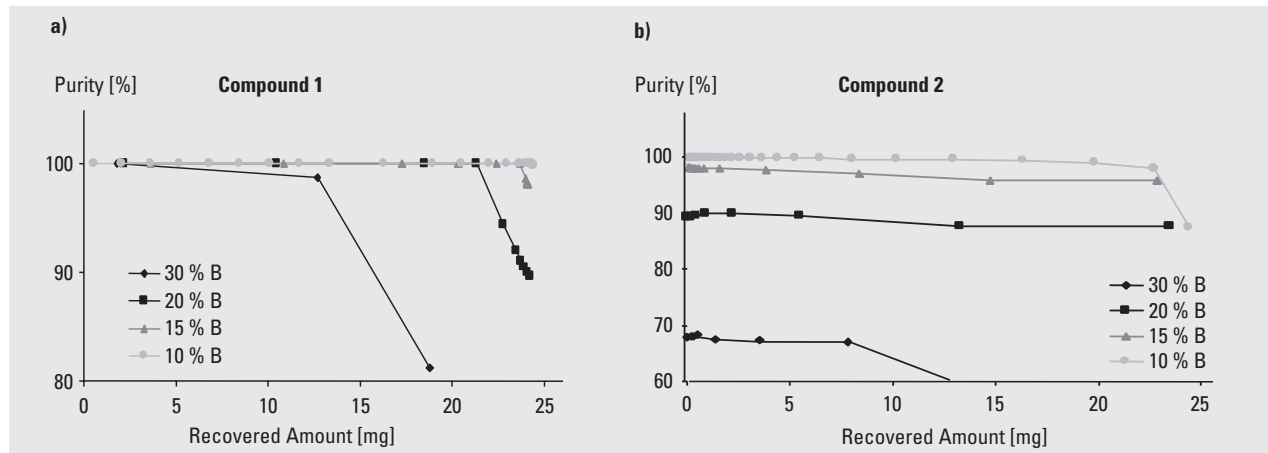


Figure 8

a) Recovery against purity for compound 1, b) recovery against purity for compound 2

Recovery and solvent consumption

Another aspect of the separation to keep in mind is the solvent consumption. Solvents for preparative LC have to be of high purity and are therefore rather expensive. Since waste disposal and environmental protection are nowadays important issues the chemist should try to avoid unnecessary solvent consumption. Figure 9 shows the recovery (purity > 90 %) against solvent usage.

It can be seen that the solvent

consumption for down to 15 % acetonitrile in the liquid phase is less than 100 ml per analysis. Using only 10 % acetonitrile in the liquid phase more than doubles the analysis run time and therefore the solvent consumption (> 250 ml per analysis). Since the results for recovery and purity are already excellent for 15 % acetonitrile in the mobile phase it is not necessary to run the analysis with less acetonitrile.

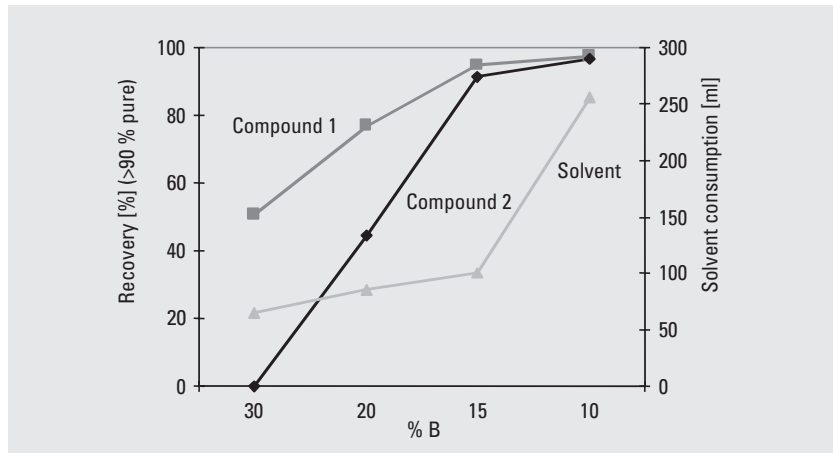


Figure 9
Recovery against solvent consumption

Conclusion

This Application Note describes the separation of two compounds from a binary mixture using the Agilent 1100 Series purification system. The purification was performed isocratically using different liquid phase compositions. The collected fractions were re-analyzed and the recovery and purity of the compounds determined. Further, the influence of the liquid

phase composition on the parameters recovery, purity and analysis run time were investigated. Also, the correlation of purity and solvent consumption, which is an important parameter regarding costs, waste disposal and environment protection, was shown.

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